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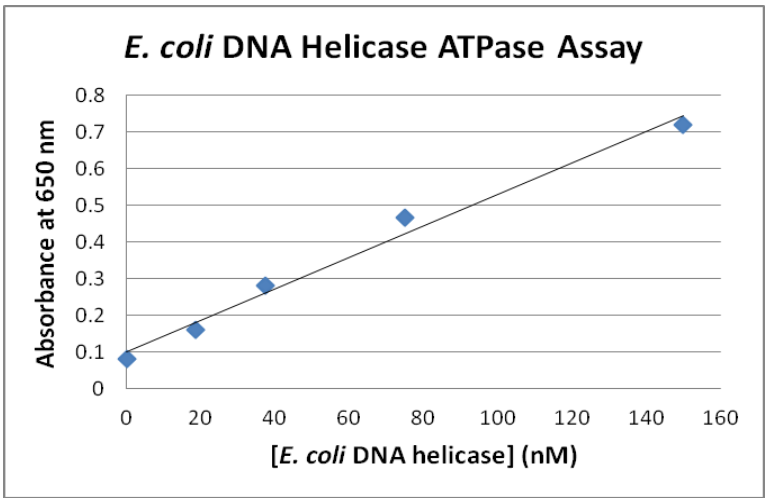
INSTRUCTIONS

ProFoldin
E.coli DNA Helicase

E. coli DNA Helicase – 100 assays Catalog No. DNAB-100EC

Protein construct: Wild-type *E. coli* DNA helicase purified from a bacterial expression system.
MW: 52 kDa
Enzyme concentration: 20 µM
Enzyme activity assay: The ATPase activity of DNA helicase is measured by using the **DNA Helicase ATPase assay Kit** (Catalog No. DNAB100K).
Storage temperature: -20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution: Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution



The ***E. coli* DNA Helicase – 100 assays (Catalog No. DNAB-100EC)** includes 33 µl of 100 x *E. coli* DNA helicase (20 µM). It is for 100 assays.

Assay Protocol using the DNA Helicase ATPase assay Kit

1. Reagent preparation:

For each 10 assay reactions,

(1) Prepare 297 µl of premix composed of 261 µl of H_2O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *E. coli* DNA helicase.

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x ATP and 3.3 µl of 100 x DNA and 26.4 µl of water.

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2. Reaction:

Mix 27 µl of the premix with 3 µl of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations for the ATPase assays of the helicases are 20 mM HEPES, pH 7.5, 20 mM potassium glutamate, 1 mM DTT, 0.005% Triton X-100, 10 mM $MgCl_2$, 250 nM DNA, 0.25 mM ATP and 200 nM DNA helicase. A negative control reaction can be the reaction mixture without addition of ATP or enzyme.

3. Detection:

Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Assay optimization for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at http://www.profoldin.com/services.html.

Reference

Nakano T., et al, Translocation and Stability of Replicative DNA Helicases upon Encountering DNA-Protein Cross-links, J. Biol. Chem. 288: 4649-4658 (2013).

Related products

DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100K
E. coli DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KE
H. influenzae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KH
S. pneumoniae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KN
P. aeruginosa DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KP
S. aureus DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KS
E. coli DNA Primase Assay Kit Plus	Catalog No. EGA100KE
H. influenzae DNA Primase Assay Kit Plus	Catalog No. HGA100KE
S. pneumonia DNA Primase Assay Kit Plus	Catalog No. PGA100KE
S. aureus DNA Primase Assay Kit Plus	Catalog No. AGA100KE

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.